REMARKS

Claims 1-34, 38, 44-46 and 48-51 are pending; claims 1-26, 33, 34, 38, 44, 46 and 48-51 have been withdrawn from consideration; claims 27-32 and 45 are rejected.

No new matter has been added. Entry of this Amendment is respectfully requested.

I. Claim Rejections Under 35 U.S.C. §112, first paragraph

At page 3 of the Office Action, claims 27-32 and 45 are rejected under 35 U.S.C. §112, first paragraph, as lacking enablement and written description support.

A. As to the enablement issue, at pages 4-5 of the Office Action the Examiner states that it would require undue experimentation in order for one of ordinary skill in the art to practice the claimed invention. As the basis for his position, the Examiner notes that there is some controversy in the literature as to whether the activity of the sodium pump can be used as a reliable basis from which to diagnose bipolar disorder. The Examiner cites to the El-Mallakh and Buss publication. The Examiner also suggests that Applicants make contradictory statements in the specification.

Applicants respectfully traverse the Examiner's position for the following reasons.

At the outset, while the Examiner has cited to publications by El-Mallakh and Buss, such publications only serve to demonstrate the state of the art prior to the instant invention (circa 1996). While there may have been some controversy concerning the reliability of using the activity of the sodium pump as a basis from which to diagnose bipolar disorder in 1996, because Applicants have convincingly demonstrated that the method of diagnosis of the present invention is functional, the cited publications should have little relevance to the enablement of the claimed method.

Similarly, the statement at page 7, lines 1-9, of the specification merely provides insight into the state of the art prior to the filing of the pending application. While one could not have expected that Na^+K^+ATPase activity could serve as a reliable basis for diagnosing bipolar disorder prior to the instant invention, because Applicants have provided convincing data of a

reliable method of diagnosing bipolar based on Na^+K^+ATPase activity, such a background statement has no bearing on the enablement of the claims.

The Examiner also refers to the statement at page 8, lines 9-13, of the specification that the membrane potential of culture cells from bipolar patients is significantly different from that of unaffected controls, and that such statement is contradictory in view of Applicants' disclosure that "it is not reliable to use transmembrane potential to serve as a basis of diagnosing bipolar disorder."

Applicants first note that the Examiner has not indicated where in the specification Applicants state that "it is not reliable to use transmembrane potential to serve as a basis of diagnosing bipolar disorder." Second, even if such a statement were made by Applicants, page 8, lines 9-13, of the specification only makes an observation concerning the difference in membrane potential between two groups of cells. It says nothing regarding the *reliability* of using this difference as a basis for diagnosing bipolar disorder.

Previous attempts to establish the sodium pump as a reliable parameter involved in bipolar disorder were unsuccessful and controversial. As such, Applicants point out in the specification that the measurement of the sodium pump was not a reliable biological marker in diagnosing bipolar disorder. On the other hand, there was enough evidence to show that membrane potential could be used for diagnostic purposes. However, direct measurement of the membrane potentials in individual patients was not reliable enough for use in diagnosis of individual patients in an economical manner. Although prior publications showed that these parameters are all involved in the pathologies of bipolar disorder, a reliable method was needed so that the diagnosis of individual patients could be accomplished using a reproducible and reliable assay at reasonable cost. This is exactly what has been accomplished in the instant application.

In light of these facts, the instant specification points out that the research results of the previous investigators, including El-Mallakh et al (1996) and Tamara et al (1996), could not serve as diagnostic markers. Moreover, while the results obtained by El-Mallakh et al (1996) using leukocytes from hospitalized patients showed some encouraging results, the follow on

results from the same group, Tamara et al (1996), using cultured lymphoblast, showed no difference. It was therefore concluded that membrane potential could not be used as a diagnostic marker.

Furthermore, the measurement of membrane potentials has to account for variations in dye concentration, cell concentration, pH, temperature and other experimental variations. Applicants overcame these hurdles and difficulties by perfecting the claimed means of measuring the membrane potentials of live cells extracted from hospitalized patients as well as euthymic patients and undiagnosed patents, and by comparing them with both normal controls and mentally ill patients without bipolar disorder. This was accomplished using a ratio method by which the errors in measurements, including variations in dye loading, cell concentration, temperature and other experimental errors, could be cancelled out by simultaneously measuring the membrane potentials of the same cell populations both in a reference buffer (regular buffer) and novel test buffers specifically designed for this purpose. The efforts to develop the test buffers are extensively discussed in the application. (At this point it may be pointed out that the contradicting result obtained by El-Mallikh's group was in most part due to their method of measurement being fraught with the errors in measurements pointed out above). By developing the ratio method and the required test buffers, Applicants have eliminated these limitations for the use of membrane potentials as a diagnostic marker for bipolar disorder.

1. Rational for the "Ratio Method"

A ratio metric dye that would compensate for variations in dye loading, cell number, temperature and other experimental errors did not exist for membrane potential measurements prior to the instant invention. Therefore, Applicants devised a ratio method that could reduce experimental errors. Applicants' approach is based on the following considerations:

The relationship between fluorescence intensity and membrane potential can be stated as

$$I = C V$$

where I is the fluorescence intensity, V is the membrane potential, and C is the proportionality constant. The proportionality constant C depends upon dye loading, cell concentration, temperature and other experimental errors including photo bleaching, variations in probe loading

and retention, as well as by instrumental factors such as illumination stability and sensitivity of the detector. By simultaneously performing experiments in two different buffers (regular and K+-free), C may be canceled out as shown below:

$$I_1 = CV_1 \qquad (2)$$

for the first measurement, and:

$$I_2 = CV_2 \qquad (3)$$

for the second measurement.

If a ratio between equations (3) and (2) is obtained, the result is:

$$\frac{I_2}{I_1} = \frac{V_2}{V_1} \qquad (4)$$

Fluorescence ratio measurements eliminate distortions of data caused by photo bleaching and variations in probe loading and retention, as well as by instrumental factors such as illumination stability and sensitivity of the detector. The ratio, therefore, measures differences in the potentials among samples eliminating all the errors (please see the enclosed publication by Applicants, *J. Affect. Disorders*, 2006, *in press*).

The Examiner's statement that "the measurement of membrane potential in cells from unaffected control individuals would be difficult without knowing the activity of sodium pump" is not correct. As described in the pending application, "in view of the previous studies on the possible involvement of the Na^+K^+ ATPase in bipolar disorder, one would not expect Na^+K^+ ATPase activity to serve as a reliable basis for diagnosing bipolar disorder in an individual patient, because measurements of Na^+K^+ ATPase activity are highly variable. Similarly, one would not expect trans-membrane potential to serve as a reliable basis for diagnosing bipolar disorder in an individual patient, because measurements of trans-membrane potential are highly variable" (page 7, para. 1). The membrane potentials depend on many factors including potassium channels, pH, ionic gradients and sodium pump activity among others. It is not true that one has to know sodium pump activity in order to measure membrane potentials. The measurement of membrane potentials is independent of sodium pump activity. The example

cited by the Examiner (De Fusco et al, 2003) is not applicable to the present invention. By developing the ratio method and the required test buffers of the present application, Applicants have eliminated these limitations for the use of membrane potentials as a diagnostic marker for bipolar disorder.

2. Validation of test results

The test results presented in the instant application were validated by means of a rigorous clinical trial in which experienced psychiatrists used all available diagnostic techniques including DSM IV, patient response to bipolar medications and patient feelings of well being. This is readily understood by one who is well versed in the art (please also see the enclosed publication by Applicants, *J. Affect. Disorders*, 2006, *in press*). Applicants also used state of the art statistical procedures to evaluate the sensitivity and specificity of this test and compared them with state of the art values for other diseases as discussed in the pending application.

3. Support in the specification

Applicants also note that the specification fully supports the method recited in the rejected claims. In particular, Example 4 provides a detailed description of using the method as recited in claim 27, using the elected species of ethacrynate. Examples 5-7 provide a detailed description of using the method as recited in claims 27, using the non-elected species. The results from the experiments demonstrate that the claimed method produces a statistically significant, reliable result in the diagnosis of bipolar disorder.

Moreover, the experiments described in Examples 8-10 provide the results of further, real world applications of the claimed method, again demonstrating that the claimed method produces a statistically significant, reliable result in the diagnosis of bipolar disorder.

As set forth in section 2164.04 of the Manual of Patent Examining Procedure (MPEP):

> A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. (emphasis added)

Applicants have provided a detailed description of the invention, the manner in which the invention can be used, and the results from numerous experiments demonstrating that the claimed method can be used in a statistically significant, reliable manner. While the art cited by the Examiner published ten years ago might have suggested that a method of bipolar disorder diagnosis based on sodium pump activity could not be conducted in a reliable manner, Applicants have overcome the obstacles noted in the art and have clearly established that such a method of diagnosis can serve as a reliable basis for diagnosing bipolar disorder.

Furthermore, Applicants note that because the claims are limited to a method of diagnosing only bipolar disorder, using a comparison of one specific physiological characteristic (membrane potential) using compounds with a specific activity (altering Na^+K^+ATPase activity), the claims are not broad. Further, the nature of the invention is a relatively straightforward assay that can be conducted in a basic laboratory, the state of the prior art is high in that one of ordinary skill in the art would be able to perform the claimed method, and the level of skill required to conduct the claimed method is relatively low. While the art cited by the Examiner suggests that the level of predictability in the art is low, Applicants have clearly and repeatedly demonstrated success with the claimed method. In addition, Applicants provide a sufficient amount of direction to practice the claimed method, as well as a number of working examples. Finally, in view of the disclosure in the application and the number of working examples, the quantity of experimentation needed to make or use the invention based on the content of the disclosure would be very low.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this portion of the rejection.

B. At page 6 of the Office Action, the Examiner states that the method recited in claim 30 is not described in the specification.

Applicants note that claim 30 is directed to the primary method of claim 27, but further recites that the cells exposed to a compound that alters Na^+K^+ATPase activity are incubated in

the absence of K^+, while cells not exposed to a compound that alters Na^+K^+ATPase activity are incubated in the presence of K^+.

Applicants respectfully note that the Examiner appears to have overlooked the description at page 27, line 35, through page 28, line 3, wherein there is a specific description of the method recited in claim 30, in conjunction with the method recited in claim 27.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this portion of the rejection.

C. At page 6 of the Office Action, the Examiner also states that the specification is only enabling for blood cells and other cells easily accessible or obtainable from patients, and that the specification does not reasonably provide enablement for other types of cells difficult or impossible to obtain from a patient, such as nerve cells from brain or adult stem cells. The Examiner states that the specification does not teach how to obtain such cells, and therefore a person of ordinary skill in the art would not have a reasonable expectation to use the claimed method.

Applicants note that MPEP section 2164.08(b) states:

> The presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled. The standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984) (prophetic examples do not make the disclosure nonenabling).
>
> Although, typically, inoperative embodiments are excluded by language in a claim (e.g., preamble), the scope of the claim may still not be enabled where undue experimentation is involved in determining those embodiments that are operable. A disclosure of a large number of operable embodiments and the identification of a single inoperative embodiment did not render a claim broader than the enabled scope because undue experimentation was not involved in determining those embodiments that were operable. *In re Angstadt*, 537 F.2d 498, 502-503, 190 USPQ 214, 218 (CCPA 1976). However, claims reading on significant numbers of inoperative embodiments would render claims nonenabled when the specification does not clearly identify the operative embodiments and undue experimentation is involved in determining

> those that are operative. *Atlas Powder Co. v. E.I. duPont de Nemours & Co.*, 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984); *In re Cook*, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1971).

The present application teaches that in a preferred embodiment, the claimed methods may use lymphoblasts, whole blood cells, lymphocytes and erthrocytes. Further, the Examples of the present invention use lymphoblasts and whole blood cells. Given this disclosure, is clear that a skilled person could easily determine which embodiments of the invention are practically operative with expenditure of no more effort than is normally required in the art. Indeed, as the specification teaches operative embodiments using lymphoblasts and whole blood cells, the skilled artisan would readily recognize which embodiments are readily operative.

While it may be difficult to obtain some cell types, it would not be impossible to do so as the Examiner suggests. One of ordinary skill would understand how to obtain each of the cell types. While some of the cells might only be obtained through dissection of the brain, such cells could be obtained if necessary. For example, a diagnosis of bipolar disorder might be needed after the death of a patient in order to establish a medical condition in a court proceeding or an insurance claim.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this portion of the rejection.

II. Claim Rejections Under 35 U.S.C. §112, second paragraph

At page 7 of the Office Action, claims 27-32 and 45 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite.

A. The Examiner states that the term "patient" is unclear as it could encompass non-human animals.

Included herewith is an amendment to the claims to more clearly recite that which Applicants regard as the invention. In view of the amendment to the claims, the claims are definite and Applicants respectfully request reconsideration and withdrawal of this rejection.

B. The Examiner states that the term "cells" is unclear because it is not clear whether the cells are *in vivo* or in culture. The Examiner also states that there is no active step of how to obtain the cells from a patient.

Included herewith is an amendment to the claims to more clearly recite that which Applicants regard as their invention. In particular, the claims have been amended to clarification that cell incubations are performed *in vitro*.

As to recitation of a step whereby the cells are obtained from the patient, Applicants respectfully assert that such a step is not required to be recited in the claim. For example, the cells could be obtained through means other than by the person performing the method recited in the claims. Such means could include a blood sample drawn by a doctor that is sent to a testing laboratory where the recited method is performed.

In view of the amendment to the claims, the claims are definite and Applicants respectfully request reconsideration and withdrawal of this rejection.

C. The Examiner states that the terms "significantly" and "significant" in claim 27 are relative terms that render the claim indefinite.

Applicants respectfully assert that the claims are definite and that the skilled artisan would readily understand the meaning of the terms "significantly" and "significant." The "significance" is determined by a statistical analysis. For example, one in twenty with a "p" value of 0.05 or less is generally accepted as significant. For the data presented in the pending application in the form of statistical tables (see Tables 5, 7 & 9), the p values are much less than 0.001. This would be considered highly significant by those well versed in the art (see, e.g., Basic and Clinical Biostatistics by Beth Dawson and Robert Trapp, McGraw-Hill Publication, 3rd edition, 2000).

In view of these comments, Applicants respectfully request reconsideration and withdrawal of this rejection.

D. The Examiner states that there is no description of the term "bipolar I disorder" recited in claim 45 to be found in the specification, and therefore the term is indefinite.

Applicants respectfully assert that the skilled artisan would understand what is meant by bipolar I disorder. At page 2, line 1-4, of the specification the DSM-IV is described. The skilled artisan would understand that a detailed description of bipolar I disorder may be found in this publication. See also Mondimore, F.M. "Bipolar Disorder - A guide for patients and families, a Johns Hopkins Press Health book, Johns Hopkins University Press, Baltimore MD, 1999).

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

E. The Examiner states that the phrase "...one or more people known to (not) have said bipolar disorder..." is indefinite because it is not clear whether such people have been diagnosed based on the DSM-IV or other means.

Applicants respectfully assert that the skilled artisan would understand that bipolar disorder can be diagnosed by several means, and that the lack of recitation of the means by which the diagnosis is achieved does not render the claims indefinite.

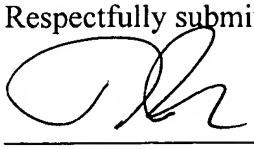
Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

III. Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



Drew Hissong
Registration No. 44,765

SUGHRUE MION, PLLC
Telephone: (202) 293-7060
Facsimile: (202) 293-7860

WASHINGTON OFFICE
23373
CUSTOMER NUMBER

Date: March 2, 2007